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Research Article

Investigation and Typing of Human Papillomavirus in Cervical Swab Samples

Abstract

In our study, the aims are typing and investigating of Human Papillomavirus (HPV) in cervical swab samples by multiplex real time polymerase chain reaction (PCR). Besides PCR results and cytological evaluation of the samples were compared.

Cytological samples were stained with Papanicolaou and modified Bethesda system was used for definition. PCR study was performed with XpertHPV.

Hundred and seventy-eight women were included in the study. In 37 of the samples (20,7%) abnormal cytology detected (25 ASCUS, 3 AGUS, 1 ASCH, 6 LSIL, 2 HSIL) and in 141 samples (79,2%) no cytological anomaly detected. HPV16 was detected in 2 patients with LSIL (2/6) and in 2 patients with HSIL (2/2). HPV DNA was detected in 10 of 25 patients with ASCUS (40%). Out of 141 with normal cytology, in 6 samples HPV16 (4,25%), in 20 samples other HPV types, totally in 26 samples (18,4%) HPV DNA was detected. HPV DNA positivity was more in women with abnormal cytology compared to women with normal cytology ($p<0.05$). Cytological anomaly rate is 35% in women with HPV (n=40) whereas this rate in HPV negative women (n=138) was detected only 16,6% ($p<0.05$).

Our study reveals regional results on HPV type distribution in cervical samples. We believe that HPV scanning should be done for all women, because both HPV is detected more in women with abnormal cytology and HPV positive women have more abnormal cytological findings. Moreover, we believe that XpertHPV test is an advantageous test with regards to quick scan and easy use.

Introduction

Cervical cancer, which the relationship is clearly proved with Human Papillomavirus (HPV), is the fourth most common cancer of women worldwide [1]. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are identified as high risk HPV (hrHPV) types. Because there is frequently latent period of 10-20 years between HPV infection and development of cervical cancer [2], national programs, especially where cytological and HPV screening tests are both used, are very important on preventing cervical cancer and early diagnosis of precancerous lesions [3]. Particularly defining high risk types regionally is essential in terms of both vaccine studies and developing molecular tests.

In our study, investigating and typing of HPV in cervical swab samples with real time PCR are targeted. Moreover, cytological evaluation of samples was compared to the results of PCR.

Material and Methods

In our study, 178 female patients between 21-74 age range

who had examined in Dumlupinar University Evliya Celebi Training and Research Hospital Obstetrics and Gynaecology Department in 2014-2015 were included. Patients had complaints such as leukore, postcoital bleeding and pain during sexual intercourse. Permission for the study was obtained from the local ethics committee and all patients were included in the study after signing the informed consent form.

Two samples were taken from patients' endocervical canal with endocervical brush. Samples were put into liquid based solution (ThinPrep Pap Test, Hologic, USA). One of the samples was sent to the pathology laboratory for cytological examination, other sample was sent to microbiology laboratory for PCR test in no time.

Cytological samples were stained with Papanicolaou and modified Bethesda system was used for definition [4,5].

In PCR study; a multiplex real time test GeneXpert HPV (Cepheid, Sunnyvale, CA) was used which can detect qualitatively E6 (early protein) / E7 (early protein) regions of the HPV types. This test can detect 14 different high risk HPV



types. Xpert HPV can give results with 6 different channels: a) Sample adequacy control (SAC) b) P1-HPV 16, c) P2-HPV18/45 d) P3-HPV 31/33/35/52/58 e) P4-HPV 51/59 f) P5-HPV 39/56/66/68. After the samples in ThinPrep transport solution were vortexed, 1.7 ml liquid was taken from it and added to PCR cartridge and when the reaction started cartridges were put into the device (GeneXpert Cepheid, Sunnyvale, CA). In each sample, DNA purification, amplification and detection were performed in only one cartridge [6].

Statistical analyses were performed using GraphPad Prism version 6.05 (Graph Pad Software, Inc., CA, and USA). All data sets were tested for normality using Kolmogorov-Smirnov test. Data were expressed as median and interquartile range (IQRs) and non-parametric statistical tests were used since data were not normally distributed. The differences between continuous variables were analysed using Mann-Whitney U test. The differences between categorical data were analysed using Fisher's exact test. A P value <0.05 was considered statistically significant.

Result

In our study, 178 female patients, whose age average was 40.42 + 9.13 SD, were included. The ages of patients ranged from 21 to 74 years old (median and interquartile ranges, IQR: 43, 35 - 48).

In 37 (20.7%) of the samples atypical cytological findings were detected whereas in 141 (79.2%) samples cytological anomaly were not detected. According to the cytological investigation in 25 patients ASCUS (Atypical squamous cells of undetermined significance), in 3 patients AGUS (Atypical glandular cells of undetermined significance), in 1 patient ASCH (Atypical squamous cells cannot exclude HSIL), in 6 patients LSIL (Low grade intraepithelial lesion) and in 2 patients HSIL (High grade intraepithelial lesion) were detected. Ten of 25 patients, who were diagnosed with ASCUS, had HPV positive (40%) and HPV 31/33/35/52/58 positivity (n=6) was the highest. Of 3 samples diagnosed with AGUS and of 1 sample diagnosed with ASCH, HPV was not detected. HPV 16 was detected in 2 samples out of 6 samples diagnosed with LSIL and 2 samples out of 2 samples diagnosed with HSIL. In 6 samples HPV 16 (4.25%), in 10 samples HPV 31/33/35/52/58, in 8 samples HPV 39/56/66/68 and in 2 samples HPV51/59 totally in 26 samples (18.4%) HPV was detected out of 141 samples with normal cytological morphology. HPV 18/45 was not detected in any of the samples (Table 1). There is a meaningful difference statistically on the rates of HPV positivity in those with normal and abnormal cytology (respectively 18.4% and 37.8%, p=0.02, Table 2). More HPV positivity rate was detected in women with abnormal cytology than women with normal cytology symptoms.

While the cytological anomaly rate for HPV positive women (n=40) is 35% (n=14, 10 ASCUS, 2 LSIL and 2 HSIL), this rate is detected as only 16.6% (n=23, 15 ASCUS, 3 AGUS, 1 ASCH and 4 LSIL) for HPV negative women (p=0.02, Table 3).

Table 1: Results of cytological investigation and HPV PCR.

| | HPV negative | HPV 16 | HPV 31/33/35/52/58 | HPV 51/59 | HPV 39/56/66/68 |
|------------------------|--------------|--------|--------------------|-----------|-----------------|
| Normal cytology(n=141) | 115 | 6 | 10 | 2 | 8 |
| ASCUS (n=25) | 15 | 0 | 6 | 2 | 2 |
| AGUS (n=3) | 3 | 0 | 0 | 0 | 0 |
| ASCH (n=1) | 1 | 0 | 0 | 0 | 0 |
| LSIL (n=6) | 4 | 2 | 0 | 0 | 0 |
| HSIL (n=2) | 0 | 2 | 0 | 0 | 0 |

Abbreviations: ASCUS: Atypical squamous cells of undetermined significance, AGUS: Atypical glandular cells of undetermined significance, ASCH: Atypical squamous cells cannot exclude HSIL, LSIL: Low grade intraepithelial lesion, HSIL: High grade intraepithelial lesion.

Table 2: HPV positivity rates in women with normal and abnormal cytology.

| | Normal cytology (n=141) | Abnormal cytology (n=37) | P |
|-------------|-------------------------|--------------------------|-------|
| Age (years) | 43 (36 - 49) | 40 (33 - 45) | 0.03* |
| HPV (-) | 115 (% 81.5) | 23 (%62.1) | |
| HPV (+) | 26 (% 18.4) | 14 (%37.8) | 0.02* |

* A P value of less than 0.05 was considered statistically significant (Fisher Exact test was used)

Table 3: Cytological findings in HPV-positive and in HPV-negative women.

| | HPV (-) (n=138) | HPV (+) (n=40) | P |
|-------------------|-----------------|----------------|-------|
| Age (years) | 42 (34 - 46) | 45 (41 - 49) | 0.02* |
| Normal cytology | 115 (%83.3) | 26 (%65) | |
| Abnormal cytology | 23 (%16.6) | 14 (%35) | 0.02* |

* A P value of less than 0.05 was considered statistically significant (Fisher Exact test was used)

Discussion

Cervical cancer is the 4th common type of cancer for women all over the world and 85% of all cervical cancer is detected in less developed countries [1]. Eastern, Southern and Central Africa, Melanesia are high risk areas for cervical cancer. Cervical cancer in Australia, New Zealand and Western Asia is seen less compared to other regions [2]. In our country cervical cancer incidence is between 4.1%-4.5% and is the 11th most common cancer type in all age group women, 5th most common cancer type in women between 15-44 ages [7]. Role of oncogenic hrHPV types on cervical cancers and development of other precancerous lesions are obvious. Infections with hrHPV types do not always end up with cervical cancer. HPV infection in young women is very high and is usually eliminated from the body within 2 years. In persistent infections the risk of cervical cancer development emerges at the end of at least 10 years' time [2,8]. Although it differs in studies, HPV prevalence in cervical cancer is more than 85% [9-12]. HPV16 and HPV18 are HPV types which are responsible for approximately 70% of all cervical cancers [2,13]. Other hrHPV types are slightly detected in cervical cancer and precancerous lesions as there may be regional differences. In Turkey HPV16/18 positivity in cervical cancer is reported as 59%-69% [7].



Cervical cancer screening is recommended for women between the ages of 30-49 in the world and this age range can be extended according to the prevalence of cervical cancer of the country [2]. Cytology, molecular HPV tests and VIA (visual inspection analysis) methods are used for cervical cancer and precancerous lesions scanning. In our study, the presence and type of HPV in cervical swab samples were researched by real time PCR and the samples were evaluated cytology.

Cytological methods are frequently used methods in community based scanning in cervical cancer. Cytological tests are carried out by two different methods, Pap smear and Liquid Based Cytology (LBC). LBC was used in our study; the most important advantage of LBC compared to the conventional method is that sufficient amount of samples can be collected and inflammatory/blood cells can be removed from the sample. Cytological methods, especially in the countries with low and middle cervical cancer prevalence, are not useful because they require serious financial investments and suitable laboratory infrastructure as well as evaluation is subjective [2]. In recent years, an antibody called p16INK has been added to cytological tests. The E7 HPV viral oncogenes cause overexpression of p16INK. The overexpression of p16INK is a strong indicator of cervical carcinoma and high level dysplasia (CIN 2 and CIN 3) associated with hrHPV. There is no p16INK expression in normal cervical epithelium or inflammatory lesions [14]. Despite all these improvement efforts sensitivity of the cytological tests is quite low [15,16]. For that reason more sensitive methods like HPV PCR tests have been developed.

For the routine use of the HPV tests, at the present time according to the considered as gold standard and FDA approved Hibrid Capture2 (HC2, Qiagen Gaithersburg, Inc., MD, USA), Cobas 4800 HPV, Cervista HR HPV or PGMY and GP5+/GP6+ primers used PCR tests should be at least 90% sensitivity and 98% specificity especially in CIN2 and higher lesions [15,17].

In our study one of a multiplex PCR based tests XpertHPV test was used. A prototype of this test was tested in one of a study that Einstein et al. performed. They were detected that, the sensitivity of the test for CIN2 and CIN3 lesions was (90%) the same as Cobas 4800 (Roche Molecular System Inc., Alemeda, CA, USA) HPV PCR test, more than HC2 test, specificity (40%) is more than Cobas 4800 HPV PCR test and less than HC2 test [6]. In similar studies, it was found out that this test's sensitivity and specificity were equivalent with present validate PCR tests and it is recommended that XpertHPV test can be used in both CIN2 and CIN3 positive women and general population scanning [18-20].

Because Xpert HPV test is a test that gives results in approximately 1 hour, it is clear that this test can be used for rapid diagnosis. Xpert HPV is a candidate as being one of a point of care (POC) tests on HPV diagnosis [21]. Moreover, for us it is an extra advantage for this test to target the E6/E7 gene regions that are responsible for oncogenic transformation [8]. However, in cases where HPV detailed typing other than HPV16 and HPV18/45 are targeted it should be studied with other molecular methods or DNA sequence analysis should be performed.

In this study, in 37 (20,7%) of the samples atypical cytological findings were detected whereas cytological anomaly was not detected in 141 samples. The HPV prevalence of women with normal cytological findings is approximately 11-12% which varies by the region, it was found out in meta-analyses that HPV types 16, 18, 31, 52, 58 were the most common ones [12,22]. As per the data for Turkey reported by ICO (Information centre on HPV and Cancer) the HPV prevalence of women with normal cytological is 12% on average where it can vary on studies (between 4.9% - 32.1%), HPV 16/18 prevalence is 4.2% and the most common detected are HPV 16/66/59/45/18 respectively [7]. In our study, in 26 (18, 4%) samples out of 141 samples with normal cytological findings HPV DNA [HPV16 (n=6, 4.2%) and other hrHPV types (n=20, %14.1)] were detected. In 10 women with normal cytological findings HPV 31/33/35/52/58, in 8 HPV 39/56/66/68 and in 2 HPV 51/59 were detected. According to the results of our study, even HPV positivity in women with normal cytological symptoms is generally relatively high, HPV16 positivity rate was detected similar to Turkey's data.

It had been advised in the backdated studies that especially for women with normal cytology and for women with negative HPV test, it is enough to scan in 8-10 years intervals and women with abnormal cytology should be directed to HPV tests [8]. Especially scanning in 3-5 years' time for the women with normal cytological symptoms is recommended by WHO whereas they emphasises the necessity that scanning intervals should be at least 5 years (they don't recommend before 10 years) for the women with HPV negative tests [23]. HPV tests are recommended before VIA and cytology/biopsy tests in scan and treat strategies. If HPV test is detected as negative it is not recommended to do an extra test or treatment whereas if it is detected positive extra tests like VIA/cytology/biopsy tests or methods like cryotherapy are recommended [24].

In our study, HPV DNA was detected in 14 of (37,8%) 37 cervical samples with abnormal cytological findings (25 ASCUS, 3 AGUS, 1 ASCH, 6 LSIL, 2 HSIL). In 10 of 25 patients diagnosed with ASCUS was HPV positive (40%) and HPV 31/33/35/52/58 positivity was the most common (n=6, 24%). HPV16 was detected in 2 samples out of 6 that were diagnosed with LSIL and in 2 samples that were diagnosed with HSIL (n=2). The prevalence of HPV is around 70% in samples with atypical cytological symptoms in the world [12]. In women with low-grade and high-grade cervical lesions in Turkey, HPV16/18 prevalence was reported 24% and 31% respectively. HPV 16/51/53/59/52/18 were reported in low-grade lesions and HPV 16/53/31/51/18/33 were reported in high-grade lesions [7]. In our study, although HPV18 and HPV45 were not detected, HPV 16 was detected only in LSIL (2/6) and HSIL (2/2) diagnosed women. Because the number of LSIL and HSIL diagnosed patients are low we believe that giving an exact rate for HPV 16 would not be right. Especially because HPV 45 and HPV 18 were not detected in any of the samples, it is thought-provoking success of HPV vaccine which is planned for the future in our country.

In our study, the most detected cytological anomaly was ASCUS (n=25). Using HPV tests are more sensitive compared to repeated cytological tests for detecting such low grade lesions



[25]. ASCUS and LSIL diagnosed women should be evaluated with HPV tests and if HPV is detected they should be treated with colposcopy or cryotherapy [26,27].

However it is clear that hrHPV rate is 18.4% in the women with normal cytology in our study whereas the rate is 37.8% in the women with atypical cytological (ASCUS, LSIL and HSIL) findings. HPV positivity rate in women with abnormal cytology was detected more than in women with normal cytology ($p<0.05$).

Matter of doing HPV tests and cytological tests together is controversial. In a study which was done by Katki et al., 340 thousand women were scanned with both cytological and HPV tests and the risk of cervical cancer development, in 5 years for women with both test's result negative, was very low and it was detected that risk was the same in the only HPV negative women [28]. According to this and similar studies, using both tests together is unnecessary [28-30]. HPV PCR based scanning can ensure more protection by means of cervical cancer development compared to cytological scanning based programs [31]. It is recommended by many scanning programs that the cytological evaluation should be done first in HPV positive women and then if ASCUS and more lesions are found they should directed to the treatments like colposcopy [15]. In our country, as a part of cervical cancer scanning run by Ministry of Health, according national cancer control plan HPV-DNA tests had been added in August 2014 for the 30-65 years old women. Through that program HPV scanning will be done to 30-65 years old women in every 5 years. HPV positive cases will be evaluated with cytological tests [32].

In our study, cytological anomaly rate is 35% ($n=14$, 10 ASCUS, 2 LSIL and 2 HSIL) in hrHPV positive women whereas that rate is detected only 16.6% ($n=23$, 15 ASCUS, 3 AGUS, 1 ASCH and 4 LSIL) in hrHPV negative women. Cytological anomaly rate is more in HPV positive women than in the HPV negative patients ($p<0.05$).

Limitation of this study are; samples with normal cytology were much more than samples with abnormal cytology (respectively $n=141$ and $n=37$) and preponderance of low-grade atypical cytological findings like ASCUS, AGUS, ASCH and LSIL ($n=35$). Besides, the fact XpertHPV was used for the first time in Turkey and HPV types and positivity rates were shown on normal and abnormal cytological samples are advantages of our study.

According to our study, because both HPV was detected more in women with abnormal cytology and abnormal cytological findings were detected more in HPV positive women than HPV negative women, HPV scanning should be done for all women. Because there is a tight relationship between HPV and cervical cancer use of HPV PCR tests should not be seen as unnecessary in women with normal cytology. Because in our study HPV is detected as 18.4% in women with normal cytology and for us this rate is substantially important.

As a result, in our study HPV existence and typing in women with normal and atypical cytology were done with XpertHPV

which is a new HPV PCR test. We believe that this test is an advantageous test with regards to easy use and quick scan in our country's conditions.

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